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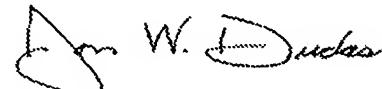
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## PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c).

**TITLE: METHOD AND APPARATUS FOR BIOWEAPON DECONTAMINATION**

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Enclosed are:

- 46 pages of specification, 7 pages of claims, and an abstract
- 4 sheet(s) of drawings.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- Yes, the name of the U.S. Government agency is the Department of Health and Human Services, National Institutes of Health.

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## METHOD AND APPARATUS FOR BIOWEAPON DECONTAMINATION

### FIELD OF THE DISCLOSURE

The present disclosure relates to the decontamination of articles that are or may  
5 be contaminated with bioweapons, particularly sporulated bioweapons, such as anthrax.

### BACKGROUND

U.S. mail, postal facilities, and government buildings have in the past been  
10 contaminated with weaponized anthrax spores, which resulted in several cases of  
bioterrorism-related inhalational anthrax infections. Because the U.S. Postal Service  
currently handles an estimated 239 billion items of mail per year, the risk is high that  
another disease outbreak will result from acts of bioterrorism. To protect the public  
health, mail and buildings actually or potentially contaminated with a sporulated  
15 bioweapon from such an attack must be thoroughly decontaminated.

One problem with the decontamination of bioterrorism sites is that anthrax and  
other bioweapon spores generally are "weaponized," which changes the spores' native  
characteristics and makes them more resistant to decontamination. While conventional  
decontamination protocols, such as exposure to chlorine dioxide, ethylene oxide,  
20 formaldehyde, or steam may be sufficient to kill many sporulated bacteria, they often  
fail to completely inactivate weaponized spores.

Furthermore, even those conventional bioweapon decontamination protocols that  
are effective on non-porous surfaces typically fail to fully decontaminate porous  
surfaces, such as paper. For instance, U.S. Patent No. 4,681,739 discloses a method for  
25 decontaminating a bacterial spore-contaminated surface that is substantially gas-  
impermeable. However, this method is ineffective at decontaminating porous surfaces,  
particularly porous surfaces that are contaminated with weaponized spores. Reliance on  
such a method may permit weaponized spores to remain viable and undetected, leading

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to possible infection and death.

#### SUMMARY OF THE DISCLOSURE

Provided herein is a method of decontaminating porous article that overcomes  
5 many of the problems of prior methods. The method is particularly effective at killing  
weaponized spores, and is especially useful when the spores are present on a porous  
article, for which prior approaches are often somewhat ineffective. The method  
includes enclosing the article in an environment, humidifying the environment to  
enhance the susceptibility of the spores to subsequent decontamination with a  
10 decontamination gas such as chlorine dioxide, reducing the pressure in the humidified  
environment to provide a deep vacuum, for example at least as low as 100 inches of  
water ( $0.25396 \text{ kg/cm}^2$ ), and then introducing into the environment a concentration of  
decontamination gas effective to decontaminate the article by killing substantially 100%  
of the spores. This method is particularly effective at decontaminating porous articles  
15 because exposing the article to a deep vacuum has been found to permit effective  
penetration of the decontamination gas deep into the porous structure of the substrate.

Also provided is an apparatus for decontaminating a porous article. The  
apparatus includes a selectively sealable decontamination chamber, a decontamination  
chamber humidifier, a source of chlorine dioxide gas in fluid communication with the  
20 decontamination chamber, and a decontamination chamber vacuum generator.

The foregoing and other features and advantages will become more apparent  
from the following detailed description of a several embodiments.

#### BRIEF DESCRIPTION OF THE FIGURES

25 FIG. 1 is a schematic diagram of an apparatus for decontamination of a porous  
article.

FIG. 2 is a schematic diagram of a chlorine dioxide generator for use in the  
apparatus of FIG. 1.

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**FIG. 3** is a diagram of a rigid container for use as a selectively sealable decontamination chamber in the apparatus of FIG. 1.

**FIG. 4** is a diagram of a room that can provide the selectively sealable decontamination chamber of FIG. 1.

5       **FIG. 5** is a graph showing the number of organisms recovered after exposure to 1,000 ppm ClO<sub>2</sub> following exposure to a deep vacuum.

#### **DETAILED DESCRIPTION**

10      **I. Introduction**

Disclosed herein is a method for decontaminating a porous article that is actually or potentially contaminated with spores. Unlike many conventional methods of decontamination, which often are ineffective at killing weaponized spores, particularly weaponized spores on porous substrates, the present method involves the use of one or 15 more humidification steps prior to the application of a deep vacuum, which is followed by the application of chlorine dioxide gas. The humidification step enhances the susceptibility of spores (particularly weaponized spores) to subsequent decontamination with chlorine dioxide. Application of the deep vacuum then allows the chlorine dioxide gas to penetrate the porous article more effectively. These factors act in concert to 20 ensure that the article is fully decontaminated, even when the article is porous and the spores are weaponized.

The method includes enclosing the article in an environment, humidifying the environment to enhance the susceptibility of the spores to subsequent decontamination with a decontamination gas (such as chlorine dioxide), reducing the pressure in the 25 humidified environment, for example to a vacuum pressure such as at least as low as 100 inches of water (0.25396 kg/cm<sup>2</sup>) to enhance penetration of the decontamination gas into the article, and then introducing into the environment a concentration of the decontamination gas effective to decontaminate the article by killing substantially 100%

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of the spores.

The method can be carried out using any rigid, substantially gas-impermeable chamber as a decontamination chamber, for example a rigid container, an autoclave, or a hyperbaric chamber. In some embodiments, the method is performed on a larger scale,  
5 and the decontamination chamber is a sealed room or building. In particular examples, the method includes sealing the room or building to form a sealed environment, and in even more particular examples, the method also includes reinforcing one or more windows prior to reducing the pressure in the environment to avoid implosion of the windows as the ambient pressure in the room or building is reduced.  
10 The method also includes humidifying the environment to enhance the susceptibility of the spores to subsequent decontamination with a decontamination gas, such as chlorine dioxide. In some embodiments, humidifying the environment includes increasing the relative humidity of the environment to at least as high as 90%. In particular examples, the relative humidity of the environment is raised to at least as high  
15 as 90% for a defined period of time, for example at least one hour or at least three hours.

The vacuum pressure applied to the humidified environment can be adjusted to suit the particular needs of a decontamination project. For example, in certain embodiments, the pressure in the humidified environment is reduced to a pressure even lower than 100 inches of water ( $0.25396 \text{ kg/cm}^2$ ), for example at least as low as 50  
20 inches of water ( $0.12698 \text{ kg/cm}^2$ ), or at least as low as 29 inches of water ( $0.0736484 \text{ kg/cm}^2$ ).

The concentration of the decontamination gas also can be varied to suit the needs of a particular decontamination project. For example, in some embodiments the concentration of gaseous chlorine dioxide is at least as high as 1000 parts per million,  
25 whereas in other embodiments the concentration of gaseous chlorine dioxide is at least 2500 parts per million. In some embodiments, the chlorine dioxide exposure time is adjusted. For instance, in some examples, the article is exposed to the gaseous chlorine dioxide for at least one hour, for at least three hours, or for at least six hours.

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The method can be used to decontaminate various types of articles that are actually or potentially contaminated with various types of spores. For example, in particular examples, the spore is a *Bacillus anthracis* spore. In even more particular examples, the spore is a weaponized spore. In still more particular examples, the article is paper.

In certain, particular embodiments, wherein the environment is a decontamination chamber, humidifying the environment includes increasing the relative humidity of the environment to at least 90% for at least one hour, the pressure in the humidified environment is reduced to at least as low as 29 inches of water (0.0736484 kg/cm<sup>2</sup>), the concentration of the decontamination gas (such as gaseous chlorine dioxide) is at least 1000 parts per million, and the article is exposed to the gaseous chlorine dioxide for at least one hour.

In certain other embodiments, the environment is a room, enclosing the article in an *airtight* environment involves sealing the room, humidifying the environment involves increasing the relative humidity of the environment to at least 90% for at least one hour, the pressure in the humidified environment is reduced to at least as low as 50 inches of water (0.12698 kg/cm<sup>2</sup>), the concentration of gaseous chlorine dioxide is at least 1000 parts per million, and the article is exposed to the gaseous chlorine dioxide for at least one hour.

In still other, particular embodiments, the environment is a building, enclosing the article in an *airtight* environment involves sealing the building, humidifying the environment involves increasing the relative humidity of the environment to at least 90% for at least one hour, the pressure in the humidified environment is reduced to at least as low as 50 inches of water (0.12698 kg/cm<sup>2</sup>), the concentration of the decontamination gas is at least 1000 parts per million, the article is exposed to the decontamination gas for at least one hour, and the method further comprises reinforcing a window prior to reducing the pressure in the environment.

In one particular example, the method is a method of decontaminating a porous

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article, and the method includes enclosing the article in a decontamination chamber, increasing the relative humidity in the decontamination chamber to at least 95%, reducing the pressure in the humidified decontamination chamber to at least as low as 50 inches of water ( $0.12698 \text{ kg/cm}^2$ ), and then introducing into the decontamination 5 chamber at least 1000 parts per million of the decontamination gas, thus decontaminating the article by killing substantially 100% of the spores.

In another particular example, the method is a method of decontaminating a porous article, and the method includes enclosing the article in a sealed room or building, increasing the relative humidity in the sealed room or building to at least 95%, 10 reducing the pressure in the humidified room or building to at least as low as 100 inches of water ( $0.25396 \text{ kg/cm}^2$ ), and then introducing into the room or building at least 1000 parts per million of the decontamination gas, thus decontaminating the article by killing substantially 100% of the spores.

Also disclosed herein is an apparatus for decontaminating a porous article. The 15 apparatus includes a selectively sealable decontamination chamber, a decontamination chamber humidifier, a source of decontamination gas (such as chlorine dioxide) in fluid communication with the decontamination chamber, and a decontamination chamber vacuum generator. In some embodiments, the apparatus also includes a first fluid flow path for transferring humidified gas from the decontamination chamber humidifier to 20 the selectively sealable decontamination chamber, a second fluid flow path for transferring decontamination gas from the source of the gas to the selectively sealable decontamination chamber, and a third fluid flow path for evacuating the selectively sealable decontamination chamber via the decontamination chamber vacuum generator. In some embodiments, the apparatus also includes a flow regulator in the first fluid flow 25 path, and/or a rotometer in the first fluid flow path.

In certain embodiments, the apparatus also includes a nitrogen source and a fourth fluid flow path for transferring nitrogen gas to the decontamination chamber humidifier. In some examples, the apparatus also includes a fill valve and/or a flow

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regulator in the fourth fluid flow path. In particular examples, the apparatus also includes a flow regulator in the third fluid flow path, and in other examples the apparatus also includes a ventilation valve in the second fluid flow path.

When the decontamination gas is chlorine dioxide, the chlorine dioxide source 5 can be any source of chlorine dioxide known in the art. For example, in some embodiments, the chlorine dioxide source is a chlorine dioxide generator. In particular examples, the chlorine dioxide generator is a Saf-T-Chlor™ chlorine dioxide generator.

In some embodiments, the selectively sealable decontamination chamber is a rigid container. In particular examples, the apparatus also includes a heat source for 10 providing heat to the selectively sealable decontamination chamber. Some embodiments of the apparatus also include a hygrometer for regulating humidity in the selectively sealable decontamination chamber.

In particular examples of the apparatus, the rigid container includes a heat source, a thermostat for regulating the heat source, and a hygrometer for regulating 15 humidity in the selectively sealable decontamination chamber.

The decontamination chamber can be any rigid, substantially gas-impermeable chamber, for example an autoclave, a hyperbaric chamber, a room, or a building. In certain examples where the decontamination chamber is a room or a building, the room or building is a sealed room or building. In particular examples, the room or building 20 includes a window and the window is reinforced to withstand a vacuum pressure of at least as low as 100 inches of water ( $0.25396 \text{ kg/cm}^2$ ), at least as low as 50 inches of water ( $0.12698 \text{ kg/cm}^2$ ), or at least as low as 29 inches of water ( $0.0736484 \text{ kg/cm}^2$ ).

In some examples, the apparatus also includes a heat source for providing heat to 25 the room or building, and in certain examples the apparatus also includes a hygrometer for regulating humidity in the selectively sealable decontamination chamber.

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**II. Abbreviations**

	atm	atmosphere
	cc	cubic centimeter
	cm	centimeter
5	ClO <sub>2</sub>	chlorine dioxide
	in.	inches
	Hg	mercury
	kg	kilogram
	lb	pound
10	mbar	millibar
	mL	milliliter
	mm	millimeter
	μm	micrometer
	mtorr	millitorr
15	N <sub>2</sub>	nitrogen
	N/m <sup>2</sup>	Newtons per square meter
	Pa	pascal
	PSI	pounds per square inch
	WG	water gauge

20

**III. Terms**

Unless otherwise noted, technical terms are used according to conventional usage. In order to facilitate review of the various embodiments of the invention, the following explanations of specific terms are provided:

25

**Autoclave:** a tank-like device for heating substances above their boiling point, often used to manufacture chemicals or sterilize surgical instruments. In some embodiments disclosed herein, an autoclave is used as a decontamination chamber for

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decontaminating bioweapon-contaminated articles.

*Bacillus*: a genus of bacteria whose collective features include degradation of most substrates derived from plant and animal sources, including cellulose, starch, pectin, proteins, agar, hydrocarbons, and others; antibiotic production; nitrification; 5 denitrification; nitrogen fixation; facultative lithotrophy; autotrophy; acidophily; alkaliphily; psychrophily, thermophily and parasitism. Spore formation, universally found in the genus, is thought to be a strategy for survival in the soil environment, wherein the bacteria predominate. Aerial distribution of dormant spores likely explains the occurrence of *Bacillus* species in most habitats examined.

10 There are more than 40 recognized species in the genus *Bacillus* (Bergery's Manual of Systematic Bacteriology Vol 2 (1986)). These include, but are not limited to, *B. acidocaldarius*, *B. alkalophilus*, *B. alvei*, *B. anthracis*, *B. azotoformans*, *B. badius*, *B. brevis*, *B. cereus*, *B. circulans*, *B. coagulans*, *B. fastidiosus*, *B. firmus*, *B. globisporus*, *B. insolitus*, *B. larvae*, *B. laterosporus*, *B. lenticorbus*, *B. lentus*, *B. licheniformis*, *B. 15 macerans*, *B. macquariensis*, *B. marinus*, *B. megaterium*, *B. mycoides*, *B. pantothenticus*, *B. pasteurii*, *B. polymyxa*, *B. popillia*, *B. pumilus*, *B. schlegelii*, *B. sphaericus*, *B. stearothermophilus*, *B. subtilis*, and *B. thuringiensis*. In one specific, non-limiting example, a *Bacillus* is *Bacillus anthracis*, the agent that causes Anthrax.

20 **Bacteria:** any of various prokaryotic organisms, including organisms within various phyla in the Kingdom Prokaryotae. The terms encompass all microorganisms commonly regarded as bacteria, including Mycoplasma, Chlamydia, Actinomyces, Streptomyces, and Rickettsia. The term also includes cocci, bacilli, spirochetes, spheroplasts, protoplasts, etc. **Spore-forming or sporulating bacteria** are bacteria that 25 are capable of forming spores, which are small, usually single-celled reproductive bodies that are highly resistant to desiccation and heat and are capable of growing into a new organism. Spore-forming bacteria include, but are not limited to members of the genera *Bacillus*, *Clostridium*, *Desulfotomaculans*, *Sporolactobacillus*, and *Sporosarcina*.

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**Biological weapon or bioweapon:** any of various bacteria, viruses, and toxins that is or can be dispersed deliberately to cause disease or death to humans, animals, or plants, or other biological organisms. Examples of biological weapons include *Bacillus anthracis* that causes anthrax, *Yersinia pestis* that causes plague, and *Variola major* that causes smallpox. Biological weapons also include biotoxins, which are any of various poisons produced by certain biological organisms, such as botulinum toxin, produced by the bacterium *Clostridium botulinum*, and ricin, isolated from castor oil seeds. A **sporulated bioweapon** is a bioweapon that includes spores, for example bacterial spores.

10       **Chlorine dioxide:** Chlorine dioxide gas has a greenish yellow color with a distinctive odor similar to that of chlorine. Chlorine dioxide is highly soluble in water but, unlike chlorine, chlorine dioxide does not react with water. It exists in aqueous solution as a dissolved gas. Chlorine dioxide functions as a highly selective oxidant owing to unique, one-electron transfer mechanisms, wherein it attacks electron-rich centers in organic molecules and, in the process, is reduced to chlorite ion.

15       Chlorine dioxide is an extremely effective disinfectant, which rapidly inactivates bacteria, viruses, and parasites such as Giardia and Cryptosporidium. In addition, under the correct reaction conditions, chlorine dioxide inactivates bacterial spores, for example *Bacillus anthracis* spores. High-purity chlorine dioxide gas is an excellent 20 gas-phase decontaminating agent, because chlorine dioxide gas molecules can kill aerosolized, airborne pathogens, and also can diffuse through cracks and crevices in an article or a building or room and reach any surface that might have been reached by the target pathogen.

25       A **source of chlorine dioxide** is any device that stores, releases, or produces chlorine dioxide. One type of a chlorine dioxide source is a **chlorine dioxide generator**. A chlorine dioxide generator is a device for producing chlorine dioxide gas, for example, a device that generates chlorine dioxide gas as needed. One such chlorine dioxide generator is the **Saf-T-Chlor™** chlorine dioxide generator (CDG, Bethlehem,

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PA), which uses the reaction between dilute chlorine gas and thermally stable solid sodium chlorite to generate chlorine dioxide gas on demand. This reaction produces chlorine dioxide gas (in nitrogen), free of chlorite ion, chlorate ion or molecular chlorine.

5       **Decontamination:** to rid of actual or potential contamination.

Decontamination gas: a gas effective to kill or otherwise substantially eliminate the pathogenicity of a sporulated pathogen, such as *Bacillus anthracis* spores, particularly weaponized spores. Examples of such decontamination gases include ethylene oxide, formaldehyde, and steam, but in particular disclosed embodiments, 10 chlorine dioxide is used.

**Decontamination chamber:** a reaction chamber for decontaminating articles that are actually contaminated or suspected to be contaminated with spores.

Decontamination chambers generally are capable of withstanding low atmospheric pressures, for example a pressure of at least as low as 100 (0.25396 kg/cm<sup>2</sup>), 50 15 (0.12698 kg/cm<sup>2</sup>), or even 29 inches of water (0.0736484 kg/cm<sup>2</sup>). In addition, a decontamination chamber generally is a substantially gas-impermeable environment. Decontamination chambers include, but are not limited to sealed, rigid containers, autoclaves, hyperbaric chambers, and rooms or buildings that have been sealed to prevent the influx or efflux of gas.

20       **Humidity:** a measure of the amount of moisture present in a gas. Generally, the degree of humidity is expressed as **relative humidity**, or the ratio of the amount of water vapor in a gas at a specific temperature to the maximum amount that the gas could hold at that temperature, expressed as a percentage. A completely saturated gas is said to be at 100% relative humidity, and partial saturation is designated by smaller 25 percentages, for example, 95%, 85%, 75%, 50%, or even less relative humidity.

**Humidification**, the process of increasing the relative humidity, can be accomplished, for example, by a **humidifier**. Examples of humidifiers include, but are not limited to evaporative humidifiers, steam humidifiers, and ultrasonic humidifiers. Humidity can

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be measured by a device known as a **hygrometer**.

**Hyperbaric chamber:** a chamber in which the oxygen pressure is maintained above normal for the atmosphere. Hyperbaric chambers often are room-sized chambers that are used in treating infections, breathing disorders, or carbon monoxide poisoning.

5 In some embodiments disclosed herein, a hyperbaric chamber is used as a decontamination chamber, particularly for decontaminating a porous article.

**Porous:** having pores, cracks, or crevices. A porous article admits the passage of gas or liquid into or through pores or interstices. In general, a porous article is more difficult to effectively decontaminate than a non-porous article.

10 **Pressure:** is a measure of force/area. **Atmospheric pressure** is pressure caused by the weight of the atmosphere. At sea level it has a mean value of one atmosphere but reduces with increasing altitude. Atmospheric pressure can be measured in a variety of different units, for example: one atmosphere is equivalent to 1.01295 bars,  $1.01295 \times 10^6$  dynes/cm<sup>2</sup>, 29.9213 inches of mercury, 406.86 inches of water, 1.03325 kg/cm<sup>2</sup>,  
15 1012.95 mbar,  $7.6 \times 10^5$  mtorr,  $7.6 \times 10^5$  microns of mercury,  $1.01296 \times 10^5$  Pa,  $1.01296 \times 10^5$  N/m<sup>2</sup>, 14.696 PSI, 14.696 lb/in<sup>2</sup>, 760 torr, or 760 mm mercury.

In one embodiment, pressure is created in an environment with a vacuum generator. In some embodiments, the pressure is at least as low as 100, 80, 60, 50, 40, 30, or even 29 inches of water. For comparison, a pressure of 100 inches of water is equivalent to about 0.2458 atmospheres, or about 0.25396 kg/cm<sup>2</sup>. A pressure of 80 inches of water is equivalent to about 0.19664 atmospheres, or about 0.203168 kg/cm<sup>2</sup>. A pressure of 60 inches of water is equivalent to about 0.14748 atmospheres, or about 0.152367 kg/cm<sup>2</sup>. A pressure of 50 inches of water is equivalent to about 0.1229 atmospheres, or about 0.12698 kg/cm<sup>2</sup>. A pressure of 40 inches of water is equivalent to about 0.09832 atmospheres, or about 0.101584 kg/cm<sup>2</sup>. A pressure of 100 inches of water is equivalent to about 0.07374 atmospheres, or about 0.076188 kg/cm<sup>2</sup>. And, a pressure of 29 inches of water is equivalent to about 0.071282 atmospheres, or about 0.0736484 kg/cm<sup>2</sup>.

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**Reinforcing:** as described herein, reinforcing, for example reinforcing a window, includes any method of strengthening, supporting, or protecting a window or other structural weakness in a room or building, such that the window is capable of withstanding a vacuum pressure, for example a vacuum pressure of 100 (0.25396 kg/cm<sup>2</sup>), 50 (0.12698 kg/cm<sup>2</sup>), or even 29 inches of water (0.0736484 kg/cm<sup>2</sup>).

**Rigid container:** a container that is capable of withstanding a vacuum pressure, for example a vacuum pressure of 100 (0.25396 kg/cm<sup>2</sup>), 50 (0.12698 kg/cm<sup>2</sup>), or even 29 inches of water (0.0736484 kg/cm<sup>2</sup>).

**Rotometer:** a device based on Stokes' law, for measuring the rate of fluid flow.

10 In some embodiments, a rotometer is a tapered, vertical tube having a circular cross section in which a float moves in a vertical path to a height dependent on the rate of fluid flow through the tube.

**Seal:** a substantially gas-impermeable closure. A sealed environment, sealed room, or sealed building is one in which substantially all leaks have been blocked (for example, using plastic or other sheeting, tape, and/or caulking) to form an environment that is substantially gas-impermeable.

**Spore:** A small, usually single-celled reproductive body that is highly resistant to desiccation and heat and is capable of growing into a new organism, produced especially by certain bacteria, fungi, algae, and non-flowering plants. Spores have proven to be the most durable type of cell found in nature, and in their cryptobiotic state of dormancy, they can remain viable for extremely long periods of time, perhaps millions of years. Spores do not form normally during active growth and cell division. Rather, their differentiation begins when a population of vegetative cells passes out of the exponential phase of growth, usually as a result of nutrient depletion. Typically, one spore is formed per vegetative cell. In some examples, the mature spore is liberated by lysis of the mother cell (sporangium) in which it was formed.

Mature spores have no detectable metabolism, a state that is described as cryptobiotic. They are highly resistant to environmental stresses such as high

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temperature (some endospores can be boiled for several hours and retain their viability), irradiation, strong acids, disinfectants, etc. Although cryptobiotic, they retain viability indefinitely such that under appropriate environmental conditions, they germinate into vegetative cells.

5       **Substrate or article:** a surface on which an object, for example an organism or spore, is attached. A **porous substrate or article** includes, but is not limited to a cellulose, nitrocellulose, glass, polyester, nylon, and polyethylsulphone article. One specific, non-limiting example of a porous substrate is paper. Non-porous substrates include, but are not limited to metal, glass, non-porous ceramics, and plastic.

10      **Vacuum:** an environment that has a reduced atmospheric pressure. A **vacuum generator** is a device that creates a reduced atmospheric pressure, for example in a decontamination chamber.

15      **Viable:** capable of living, developing, or germinating under favorable conditions. For example, a viable spore is capable of developing under favorable conditions.

20      **Weaponized:** weaponization or enhancement of a bioweapon is a process of creating a finely dispersed, highly concentrated, easily aerosolized, and sterilization- or decontamination-resistant spore. Weaponization changes a spore's susceptibility to decontamination regimes and makes it harder to kill.

25      Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. "Comprises" means "includes." Hence "comprising A or B" means including A, or B, or A and B. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials

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are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

***IV. Description of Several Specific Embodiments***

5       ***A. Decontamination of porous articles***

Disclosed herein are methods for decontaminating a porous article. Many known methods of bioweapon decontamination, for instance exposure to chlorine dioxide, ethylene oxide, formaldehyde, or steam, are effective at decontaminating non-porous articles, for example non-porous glass, porcelain, and metals. However, terrorist 10 activities have targeted the United States mail system, generating numerous anthrax-contaminated parcels and envelopes, as well as mail-handling equipment, furniture, office supplies, and the like. Conventional decontamination techniques are ineffective at decontaminating such porous articles because the sterilant fails to penetrate deeply enough into the pores of the articles to fully inactivate all contaminating spores.

15       By contrast, the methods disclosed herein involve subjecting the contaminated article to a deep vacuum prior to exposure to the sterilant gas. This permits the gas to penetrate the article more fully, exposing the spores contained in inner pockets and pores to the gas, which creates a greater mass transfer of gas and results in a thorough decontamination of the article. The deep vacuum is equivalent to a pressure of at least 20 as low as 100, 80, 60, 50, 40, 30, or even 29 inches of water. For comparison, a pressure of 100 inches of water is equivalent to about 0.2458 atmospheres, or about 0.25396 kg/cm<sup>2</sup>. A pressure of 80 inches of water is equivalent to about 0.19664 atmospheres, or about 0.203168 kg/cm<sup>2</sup>. A pressure of 60 inches of water is equivalent to about 0.14748 atmospheres, or about 0.152367 kg/cm<sup>2</sup>. A pressure of 50 inches of 25 water is equivalent to about 0.1229 atmospheres, or about 0.12698 kg/cm<sup>2</sup>. A pressure of 40 inches of water is equivalent to about 0.09832 atmospheres, or about 0.101584 kg/cm<sup>2</sup>. A pressure of 100 inches of water is equivalent to about 0.07374 atmospheres, or about 0.076188 kg/cm<sup>2</sup>, and a pressure of 29 inches of water is equivalent to about

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0.071282 atmospheres, or about 0.0736484 kg/cm<sup>2</sup>. The pressure employed in a particular situation can be tailored to suit any of a variety of factors, for example, the type of decontamination chamber, room, or building to be decontaminated, the porosity of the article or articles to be contaminated, the concentration of chlorine dioxide gas used, or the length of time the article is exposed to the sterilant gas.

5           *B. Decontamination of weaponized spores*

Conventional decontamination techniques, while effective at inactivating many types of bacterial spores, often are ineffective at killing weaponized spores. Among 10 other modifications, weaponized spores generally are dessicated, which makes them particularly resistant to chemical sterilizing agents. Thus, articles contaminated with dessicated spores often require decontamination with substantially more rigorous sterilization conditions (for instance, a higher sterilant concentration or longer exposure time) than do non-dessicated spores.

15          The methods disclosed herein overcome this problem by including a humidification step that enhances the susceptibility of dessicated spores to inactivation with chlorine dioxide gas. By enhancing the susceptibility of the spores to the chlorine dioxide sterilant, a lower concentration of chlorine dioxide may be used, and/or the length of exposure to the chlorine dioxide may be shortened. Humidification of the 20 spores is accomplished by pre-humidifying the article to be decontaminated in an atmosphere of controlled humidity prior to exposing the article to the chlorine dioxide gas. Generally, the degree of humidity is expressed as relative humidity, or the ratio of the amount of water vapor in a gas at a specific temperature to the maximum amount that the gas could hold at that temperature, expressed as a percentage. A completely 25 saturated gas is said to be at 100% relative humidity, and partial saturation is designated by smaller percentages, for example, 95%, 85%, 75%, 50%, or even less relative humidity.

In some examples, the humidification step is carried out at a relative humidity of

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70%, 80%, 90%, 95%, 98%, or even higher relative humidity. In some embodiments, the article is exposed to the elevated humidity for at least 15 minutes, at least 30 minutes, or at least 1, 2, 3, 5, 10, or 20 hours, or even longer. The relative humidity chosen and the duration of exposure to the relative humidity are optimized to suit a 5 particular decontamination project, and can vary depending on, for example, the type of decontamination chamber, room, or building to be decontaminated, the porosity of the article or articles to be contaminated, the concentration of chlorine dioxide gas used, or the length of time the article is exposed to the sterilant gas. In certain examples, the humidity in the decontamination chamber is raised to a relative humidity of 70%, 80%, 10 90%, 95%, 98%, or even higher during exposure of the article to the chlorine dioxide gas.

In some examples, the humidification step is carried out at room temperature (about 68°F), although lower or higher temperatures can be employed if desired. In some embodiments, the humidification step is carried out at an elevated temperature, for 15 example 75°F, 85°F, 95°F, or even higher temperatures. Although the humidification step generally is carried out using humidified air, other humid gases, such as humidified nitrogen gas, may be used.

In certain embodiments, the decontamination chamber is a rigid container, as shown in FIG. 3. Such an embodiment is particularly suited to decontaminating small 20 articles, such as mail envelopes or parcels. In such an embodiment, a suitably-sized article, for example a piece of mail or a parcel, is placed in the rigid container, and the container is sealed prior to exposing the article to chlorine dioxide gas.

In other embodiments, the decontamination chamber is an autoclave or hyperbaric chamber. Such an embodiment is particularly suited to the decontamination 25 of medium or large-sized articles. In one particular, non-limiting example, an autoclave or hyperbaric chamber is used for the decontamination of mail, either as individual pieces or as items in trays, held in baskets, or in bins. In some embodiments, the trays, baskets, or bins are placed onto wheeled racks, or transported by automated means or

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fork lifts, or transported by any other method of holding and transporting batches of mail, and the carts or forklifts are wheeled into the autoclave or hyperbaric chamber. The autoclave or hyperbaric chamber is then sealed prior to exposing the article(s) to chlorine dioxide gas.

5 In still other embodiments, the decontamination chamber is a room or a building (see FIG. 4). Such embodiments are appropriate when decontamination is desired on a large scale, and the decontamination chamber is a sealed room or building. This embodiment is particularly useful for decontaminating rooms or buildings contaminated with weaponized spores, particularly when such rooms or buildings contain porous  
10 articles, for example paper.

In particular examples, the room or building is sealed to form a sealed environment. Sealing the room or building prevents the escape of chlorine dioxide to the atmosphere. Sealing the room or building can include, but is not limited to, sealing the windows with foil-backed foam insulation, sealing cracks with expanding foam or  
15 silicone caulking, and sealing skylights, loading docks, and building openings with poly-sheeting and foil tape. In even more particular examples, one or more windows in the room or building are reinforced prior to reducing the pressure in the environment to avoid implosion of the windows as the ambient pressure in the room or building is reduced.

20

### C. Chlorine dioxide

The particular decontamination gas used in certain examples is chlorine dioxide, a relatively small, volatile and highly energetic molecule. Chlorine dioxide gas is unstable at high concentrations; generally, it is generated at the point of use.

25 Chlorine dioxide is an extremely effective disinfectant, which rapidly inactivates bacteria, viruses, and parasites such as Giardia and Cryptosporidium. Because chlorine dioxide oxidizes but does not chlorinate, chlorinated organic by-products (for example, trihalomethanes, haloacetic acids, dioxins, and furans) typically are not produced.

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Neither does chlorine dioxide produce appreciable amounts of aldehydes, ketones, ketoacids, or other problematic compounds associated with oxidation of organic matter by other, less selective means.

In addition, under the correct reaction conditions, chlorine dioxide inactivates 5 bacterial spores, for example *Bacillus anthracis* spores. High-purity chlorine dioxide gas is an excellent gas-phase decontaminating agent, because chlorine dioxide gas molecules can kill aerosolized, airborne pathogens, and also can diffuse through cracks and crevices in an article or a room or building and reach any surface that might have been reached by the target pathogen.

10 The chlorine dioxide gas may be prepared by any of the methods known in the art. One such method involves passing a stream of air-diluted chlorine gas or nitrogen-diluted chlorine gas at a metered rate through a column of finely divided sodium chlorite, and into a partially evacuated chamber. This procedure is described more fully in Grubitsch *et al.*, *Monatsh.*, Vol. 93, p. 246 (1962).

15 Another method of preparing chlorine dioxide gas is the reaction of sodium chlorite solutions in the presence of acids. In one embodiment, a dilute solution of aqueous potassium persulfate is treated with a dilute solution of aqueous sodium chlorite at ambient temperatures (20-30°C) in a closed reaction vessel. This method is discussed more fully in Rosenblatt *et al.*, *J. Org. Chem.*, 28, 2790 (1963).

20 In some embodiments, the chlorine dioxide gas is delivered via a chlorine dioxide source, which can be any device that stores, releases, or produces chlorine dioxide. One type of a chlorine dioxide source is a chlorine dioxide generator. A chlorine dioxide generator is a device for producing chlorine dioxide gas, for example, a device that generates chlorine dioxide gas as needed. One such chlorine dioxide 25 generator is the CDG Saf-T-Chlor™ chlorine dioxide generator (CDG, Bethlehem, PA), which uses the reaction between dilute chlorine gas and thermally stable solid sodium chlorite to generate chlorine dioxide gas on demand:

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This reaction produces chlorine dioxide gas (in nitrogen), free of chlorite ion, chlorate ion or molecular chlorine.

5 CDG Gas: Solid chlorine dioxide generators are available in at least two sizes: bench-scale generators for smaller scale applications and plant-scale generators, which are useful for providing chlorine dioxide in amounts sufficient to decontaminate large areas, or for the routine decontamination of large volumes of mail.

10 In some embodiments, the chlorine dioxide gas is delivered to the decontamination chamber in the form of a gaseous mixture of chlorine dioxide and an inert carrier gas. One specific, non-limiting example of an inert carrier gas is nitrogen gas. In some embodiments, the chlorine dioxide gas is delivered to the decontamination chamber in the form of a gaseous mixture of chlorine dioxide and air.

15 The concentration of chlorine dioxide can be varied to suit the needs of a particular decontamination project. For example, in some embodiments the concentration of gaseous chlorine dioxide is at least as high as 1,000 parts per million, whereas in other embodiments the concentration of gaseous chlorine dioxide is at least as high as 1,500, 2,000, 2,500, 3,000, or 3,500 parts per million, or even higher. In some embodiments, the chlorine dioxide exposure time is adjusted. For instance, in 20 some examples, the article is exposed to the gaseous chlorine dioxide for at least one hour, for at least three hours, or for at least six hours. The particular concentration of chlorine dioxide in the carrier gas selected for use is a function of several factors, including the inherent ability of the particular spores to resist decontamination by chlorine dioxide, the duration of exposure to the chlorine dioxide gas, the degree to 25 which the spores are dessicated, the humidity to which the article has been exposed during the humidification step, the duration of the humidification step, and the relative humidity of the chlorine dioxide/carrier gas.

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*D. Apparatus for decontamination of porous articles*

Also disclosed herein is an apparatus for decontaminating porous articles. One embodiment of the apparatus 10 is shown in FIG. 1. Apparatus 10 includes a nitrogen source 12 in fluid communication with a decontamination chamber humidifier 14 and a first fluid flow path 16 for transferring nitrogen gas to decontamination chamber humidifier 14. First fluid flow path 16 includes a fill valve 18, which permits the addition of gas or liquid to first fluid flow path 16, and a flow regulator 20, which regulates flow of the nitrogen gas to decontamination chamber humidifier 14.

Decontamination chamber humidifier 14 is in fluid communication with a rotometer 22 via a second fluid flow path 24. Rotometer 22 is in fluid communication with one inlet of a T junction 26 via a third fluid flow path 28. Third fluid flow path 28 includes a flow regulator 30, which regulates flow of the humidified nitrogen gas to T junction 26.

Apparatus 10 also includes a source of chlorine dioxide gas 32, which is in fluid communication with the second inlet of T junction 26 via fourth fluid flow path 34. The outlet of T junction 26 is in fluid communication with a selectively sealable decontamination chamber 36 via a fifth fluid flow path 38. Fifth fluid flow path 38 includes a flow regulator 40, which regulates flow of a mixture of chlorine dioxide gas and nitrogen gas from T junction 26 to selectively sealable decontamination chamber 36, and a ventilation valve 42, which permits the influx or efflux of gas from fifth fluid flow path 38.

Selectively sealable decontamination chamber 36 accommodates one or more articles for decontamination, and includes a lid 44 that can be opened or closed as desired. When closed, lid 44 forms a gas-tight closure. Selectively sealable decontamination chamber 44 is in fluid communication with a vacuum generator 46 via a sixth fluid flow path 48. Sixth fluid flow path 48 includes a flow regulator 50, which regulates flow of exhaust gas from selectively sealable decontamination chamber 36 to vacuum generator 46.

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In operation, an article in need of decontamination is enclosed in selectively sealable decontamination chamber 36. Lid 44 is then sealed to form a gas-impermeable seal, and humidification of decontamination chamber 36 is initiated. Nitrogen gas from nitrogen source 12 flows through first fluid path 16 to decontamination chamber 5 humidifier 14, where the nitrogen gas is humidified. Flow regulator 20 regulates the pressure of the nitrogen gas in first fluid flow path 16.

Humidified nitrogen gas flows from decontamination chamber humidifier 14 to rotometer 22 through second fluid flow path 24. Humidified nitrogen gas then flows from rotometer 22 through third fluid flow path 28 to the first inlet of T junction 26, out 10 the outlet of T junction 26, and through fifth fluid flow path 38 to selectively sealable decontamination chamber 36. The article is incubated in the humidified nitrogen gas for a predetermined time. The relative humidity of the humidified nitrogen gas and the duration of incubation are determined based on the particular characteristics of the article being decontaminated, including, but not limited to, the porosity of the article, 15 the inherent ability of potential or actual contaminating spores to resist decontamination by chlorine dioxide, the concentration of chlorine dioxide gas to be used, the degree to which potential or actual contaminating spores are dessicated, and the relative humidity of the chlorine dioxide/nitrogen gas mixture to be used.

After incubation, the humidified nitrogen gas is exhausted from selectively 20 sealable decontamination chamber 36 by vacuum generator 46 through sixth fluid flow path 48. Vacuum generator 46 continues to remove gas from selectively sealable decontamination chamber 36 until a desired vacuum pressure is achieved in selectively sealable decontamination chamber 36, for example a vacuum pressure equivalent to at least as low as 100, 50, or 29 inches of water.

25 Following the humidification step, a decontamination step begins. Nitrogen gas from nitrogen source 12 flows through first fluid path 16 to decontamination chamber humidifier 14, where the nitrogen gas is humidified. Humidified nitrogen gas flows from decontamination chamber humidifier 14 to rotometer 22 through second fluid flow

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path 24. Humidified nitrogen gas then flows from rotometer 22 through third fluid flow path 28 to the first inlet of T junction 26. Chlorine dioxide gas from source 32 passes from source of chlorine dioxide gas 32 through fourth fluid flow path 34 to the second inlet of T junction 26. The chlorine dioxide gas combines with the humidified nitrogen gas in T junction 26 to form a chlorine dioxide/nitrogen gas mixture with a desired chlorine dioxide concentration, for instance 1,000 ppm or 2,500 ppm chlorine dioxide gas in humidified nitrogen gas. The particular concentration of chlorine dioxide in the carrier gas selected for use is a function of several factors, including, but not limited to, the porosity of the article, the inherent ability of the particular spores to resist decontamination by chlorine dioxide, the duration of exposure to the chlorine dioxide gas, the degree to which the spores are dessicated, the humidity to which the article has been exposed during the humidification step, the duration of the humidification step, and the relative humidity of the chlorine dioxide/nitrogen gas mixture.

The chlorine dioxide/nitrogen gas mixture then flows from the outlet of T junction 26 into selectively sealable decontamination chamber 36 via fifth fluid flow path 38. The article is incubated in the chlorine dioxide/nitrogen gas mixture for a predetermined time, which is chosen based on a number of factors, including, but not limited to, the porosity of the article, the inherent ability of the particular spores to resist decontamination by chlorine dioxide, the concentration of chlorine dioxide gas, the degree to which the spores are dessicated, the humidity to which the article has been exposed during the humidification step, and the relative humidity of the chlorine dioxide/nitrogen gas mixture.

After the appropriate incubation period, the chlorine dioxide/nitrogen gas mixture is then evacuated from selectively sealable decontamination chamber 36 by decontamination chamber vacuum generator 46 via sixth fluid flow path 48. Flow regulator 50 regulates the pressure of the chlorine dioxide/nitrogen gas mixture in sixth fluid flow path 48.

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*E. Chlorine dioxide generator*

In some embodiments, source of chlorine dioxide gas 32 is a CDG Saf-T-Chlor™ chlorine dioxide gas generator 50, as shown in FIG. 2. CDG chlorine dioxide generator 50 includes a sodium chlorite cartridge 52 in fluid communication with a first inlet of first T junction 54 via first fluid flow path 56. First fluid flow path 56 includes 5 a pressure regulator 58 and an on/off valve 60.

CDG chlorine dioxide generator 50 also includes a nitrogen tank 62 in fluid communication with a second inlet of first T junction 54 via second fluid flow path 64. Second fluid flow path 64 includes a pressure regulator 66 and an on/off valve 68.

10 The outlet of first T junction 54 is in fluid communication with a first inlet of second T junction 70 via third fluid flow path 72. A second inlet of T junction 70 is in fluid communication with a pressure gauge 74 via fourth fluid flow path 76. The outlet of second T junction 70 is in fluid communication with a sodium chlorite cartridge 80 via a fifth fluid flow path 82. Fifth fluid flow path 82 includes a flow meter 84 and a 15 control valve 86. Chlorine dioxide gas from sodium chlorite cartridge 80 leaves CDG chlorine dioxide generator 50 via sixth fluid flow path 88.

To generate chlorine dioxide gas, on/off valve 60 is opened, and a mixture of chlorine and nitrogen gas is transferred from chlorine/nitrogen tank 52 to the first inlet of first T junction 54 via first fluid flow path 56.

20 On/off valve 68 is also opened, and nitrogen gas is transferred from chlorine/nitrogen tank 62 to the second inlet of first T junction 54 via second fluid flow path 64. Pressure regulator 58 regulates the pressure of the gas in second fluid flow path 64. The chlorine/nitrogen gas mixture combines with nitrogen gas in first T junction 54 to form a gas mixture. The gas mixture flows from the outlet of first T 25 junction 54 to the first inlet of second T junction 70 via third fluid flow path 72. Pressure gauge 74 measures the pressure of the gas mixture via the second inlet of second T junction 70 and fourth fluid flow path 76.

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The gas mixture is then transferred from second T junction 70 to sodium chlorite cartridge 80 via fifth fluid flow path 82, where it reacts with the sodium chlorite in sodium chlorite cartridge 80 to form chlorine dioxide gas. Flow meter 84 regulates the pressure of the gas mixture in fifth fluid flow path 82, and control valve provides a mechanism for interrupting gas flow through fifth fluid flow path 82, if needed. The chlorine dioxide gas flows from sodium chlorite cartridge 80 and exits chlorine dioxide generator 50 via sixth fluid flow path 88.

*E. Decontamination chambers*

10 The selectively sealable decontamination chamber 36 described above (FIG. 1) can be any rigid, substantially gas-impermeable chamber, for example a rigid container, an autoclave, a hyperbaric chamber, a room, or a building. In one embodiment, selectively sealable decontamination chamber 36 is a rigid container 90, as shown in FIG. 3. The rigid container 90 includes a reaction vessel 92 that has a sealable opening 94 and a lid 96 for sealing the sealable opening 94. Reaction vessel 92 is supported by a stand 98, which includes a heat source 100 for providing heat to reaction vessel 92.

Reaction vessel 92 is supported by a stabilizing collar 102. Lid 94 includes a first sealable port 104 and a second sealable port 106. A thermometer or a hygrometer can be introduced into reaction vessel 92 via first sealable port 104 and/or second sealable port 106. Lid 94 also includes a third sealable port through which gas and liquid can be introduced to and removed from reaction vessel 92.

In operation, a suitably-sized article, for example a piece of mail or a parcel, is placed in reaction vessel 92, and sealable opening 94 is sealed using lid 96. Humidified gas is added to reaction vessel 92 via third sealable port 108, and the article is incubated in the humidified gas for a predetermined period of time. The relative humidity of the humidified gas and the duration of incubation are determined based on the particular characteristics of the article being decontaminated, including, but not limited to, the porosity of the article, the inherent ability of potential or actual contaminating spores to

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resist decontamination by chlorine dioxide, the concentration of chlorine dioxide gas to be used, the degree to which potential or actual contaminating spores are dessicated, and the relative humidity of the chlorine dioxide gas to be used.

The humidified gas is then evacuated via third sealable port 108, generating a vacuum pressure of at least as low as 100 inches of water. Chlorine dioxide gas is then added to reaction vessel 92 via the third sealable port 108, and the article is incubated in the chlorine dioxide gas for a predetermined time. The particular concentration of chlorine dioxide gas selected for use is a function of several factors, including, but not limited to, the porosity of the article, the inherent ability of potential or actual 5 contaminating spores to resist decontamination by chlorine dioxide, the duration of exposure to the chlorine dioxide gas, the degree to which potential or actual 10 contaminating spores are dessicated, the humidity to which the article has been exposed during the humidification step, the duration of the humidification step, and the relative humidity of the chlorine dioxide gas. The chlorine dioxide gas is then evacuated via 15 third sealable port 108, lid 96 is opened, and the article is removed from reaction vessel 92 through sealable opening 94.

In another embodiment, as shown in FIG. 4, selectively sealable decontamination chamber 36 is a room 110. Room 110 includes an influx channel 112 for transferring gas into room 110, and an efflux channel 114 for transferring gas out of 20 room 110. Influx channel 112 and efflux channel 114 pass through a doorway 116 that is sealed with a vapor barrier 118. Room 110 also includes a window 120 that is sealed with a vapor barrier 122 and reinforced with a reinforcing panel 124.

In operation, humidified gas is transferred into room 110 via influx channel 112. Room 110 is then incubated in the humidified gas for a predetermined period of time. 25 The relative humidity of the humidified gas and the duration of incubation are determined based on the particular characteristics of room 110, including, but not limited to, the porosity of articles and furnishings in room 110, the inherent ability of potential or actual contaminating spores to resist decontamination by chlorine dioxide,

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the concentration of chlorine dioxide gas to be used, the degree to which the potential or actual contaminating spores are dessicated, and the relative humidity of the chlorine dioxide gas to be used.

The humidified gas is then evacuated from room 110 via the efflux channel 114,  
5 creating a vacuum pressure of at least as low as 100 inches of water. Chlorine dioxide gas is then transferred into room 110 via influx channel 112, and room 110 is incubated in the chlorine dioxide gas for a predetermined period of time. The particular concentration of chlorine dioxide gas selected for use is a function of several factors, including, but not limited to, the porosity of articles and furnishings in room 110, the  
10 inherent ability of potential or actual contaminating spores to resist decontamination by chlorine dioxide, the duration of exposure to the chlorine dioxide gas, the degree to which potential or actual contaminating spores are dessicated, the humidity to which the article has been exposed during the humidification step, the duration of the  
humidification step, and the relative humidity of the chlorine dioxide gas. The chlorine  
15 dioxide gas is then evacuated from room 110 via efflux channel 114.

## EXAMPLES

**Example 1: Decontamination of weaponized spores with high-purity chlorine  
20 dioxide gas**

This example demonstrates that a concentration of 10,000 parts per million (ppm) chlorine dioxide gas is an effective sterilant for paper contaminated with weaponized spores.

Paper swabs (n=16) contaminated with  $2.0 \times 10^8$  weaponized spores were  
25 exposed to 10,000 ppm ClO<sub>2</sub> for four hours. Swabs were cultured under permissive culture conditions (15 hour incubation in thioglycollate broth) to determine whether the weaponized spores were viable following the decontamination protocol. Out of 16 swabs exposed to the decontamination protocol, none showed viable spores following

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decontamination. Thus, a concentration of 10,000 parts per million (ppm) chlorine dioxide gas is an effective sterilant for paper contaminated with weaponized spores.

**Example 2: Effect of pre-humidification on decontamination efficacy**

5 This example demonstrates that following a pre-humidification step carried out at 95% relative humidity and 95 °F for 1-3 hours, a concentration of 10,000 parts per million (ppm) chlorine dioxide gas is an effective sterilant for both conventional biological indicator spores and weaponized spores.

10 Paper swabs contaminated with  $2.0 \times 10^8$  weaponized spores (n=2),  $10^{10}$  weaponized spores (n=2), or  $10^6$  conventional biological indicator spores (n=2) were enclosed in envelopes and pre-humidified at 95% relative humidity and 95 °F for 1-3 hours. They were then exposed to 10,000 ppm ClO<sub>2</sub> for four hours. Swabs were cultured under permissive culture conditions (15 hour incubation in thioglycollate broth) to determine whether the spores were viable following the decontamination 15 protocol. None of the swabs showed viable spores following decontamination. (See Table 1.)

**Table 1: Effect of pre-humidification on decontamination efficacy**

Humidification time	1 hour	2 hours	3 hours
2 $\times 10^8$ Weaponized Spores Per Swab	0/2	0/2	0/2
10 <sup>10</sup> Weaponized Spores Per Swab	0/2	0/2	0/2
10 <sup>6</sup> Conventional Biological Indicator Spores Per Swab	0/2	0/2	0/2
Positive Control	$1.7 \times 10^8$	$1.7 \times 10^8$	$1.7 \times 10^8$

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**Example 3: Effect of gas concentration on decontamination efficacy**

This example demonstrates that following a pre-humidification step carried out at 95% relative humidity and 95 °F for 1.5 hours, a concentration of 1,000 parts per million (ppm) chlorine dioxide gas is an effective sterilant for both conventional biological indicator spores and weaponized spores.

Paper swabs contaminated with  $2.0 \times 10^8$  weaponized spores,  $10^{10}$  weaponized spores, or  $10^6$  conventional biological indicator spores were enclosed in envelopes and pre-humidified at 95% relative humidity and 95 °F for 1.5 hours. They were then exposed to 2,500, 1,000, or 500 ppm ClO<sub>2</sub> for four hours. Swabs were cultured under permissive culture conditions (15 hour incubation in thioglycollate broth) to determine whether the spores were viable following the decontamination protocol. Only the swabs containing weaponized spores that were exposed to the lowest concentration of chlorine dioxide (500 ppm) showed viable spores following decontamination. (See Table 2.)

15

**Table 2: Effect of gas concentration on decontamination efficacy**

ClO <sub>2</sub> Concentration	2500 ppm	1000 ppm	500 ppm
2 x 10 <sup>8</sup> Weaponized Spores Per Swab	0/2	0/2	2/2; 1.43 x 10 <sup>3</sup>
10 <sup>10</sup> Weaponized Spores Per Swab	0/2	0/2	2/2
10 <sup>6</sup> Conventional Biological Indicator Spores Per Swab	0/2	0/2	0/2
10 <sup>6</sup> Weaponized Spores Per Swab			3/4
Positive Control	$1.7 \times 10^8$	$1.7 \times 10^8$	$1.7 \times 10^8$

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Thus, following a pre-humidification step carried out at 95% relative humidity and 95 °F for 1.5 hours, a concentration of 1,000 parts per million (ppm) chlorine dioxide gas is an effective sterilant for both conventional biological indicator spores and weaponized spores.

5

**Example 4: Comparison of biological indicators at 500 ppm ClO<sub>2</sub>**

This example demonstrates the inadequacy of using non-weaponized spores to measure the decontamination efficiency of chlorine dioxide bioweapon decontamination protocols.

10 Paper swabs contaminated with  $10^6$  weaponized spores or  $10^6$  conventional biological indicator spores were enclosed in envelopes and pre-humidified at 95% relative humidity and 95 °F for 1-3 hours. They were then exposed to 500 ppm ClO<sub>2</sub> for four hours. Swabs were cultured under permissive culture conditions (15 hour incubation in thioglycollate broth) to determine whether the spores were viable

15 following the decontamination protocol. Following decontamination, the weaponized spores were still viable, whereas the conventional biological indicator spores were not. (See Table 3.)

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**Table 3: Comparison of biological indicators at 500 ppm ClO<sub>2</sub>**

Humidification Time	1 hour	2 hours	3 hours
10 <sup>6</sup> Weaponized Spores Per Swab	Positive	Positive	Positive
10 <sup>6</sup> Conventional Biological Indicator Spores Per Swab	Negative	Negative	Negative
10 <sup>6</sup> Weaponized Spores Per Swab (positive control)	Positive	Positive	Positive
10 <sup>6</sup> Conventional Biological Indicator Spores Per Swab (positive control)	Positive	Positive	Positive

Thus, conventional, non-weaponized spores provide an inadequate assessment of the decontamination efficiency of chlorine dioxide bioweapon decontamination protocols.

5

**Example 5: Efficacy of steam sterilization in decontaminating weaponized spores versus conventional bioindicator spores**

This example demonstrates the inadequacy of steam sterilization for 10 decontamination weaponized spores.

Paper swabs contaminated with 10<sup>6</sup> weaponized spores or 10<sup>6</sup> conventional biological indicator spores were enclosed in envelopes and exposed to a steam decontamination protocol for 15 minutes at 121°C and a pressure of 20 pounds per square inch. Swabs were cultured under permissive culture conditions (15 hour

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incubation in thioglycollate broth) to determine whether the spores were viable following the decontamination protocol. Following decontamination, the weaponized spores were still viable, showing heavy growth with pellicle formation, whereas the conventional biological indicator spores showed no growth.

5 Thus, steam sterilization was ineffective at decontaminating weaponized spores.

**Example 6: Effect of deep vacuum and successive treatment cycles on decontamination efficacy**

This example shows the effect of exposure to a deep vacuum and consecutive 10 treatment cycles on decontamination efficacy.

15 Weaponized spores or conventional bioindicator spores at a concentration of  $10^{10}$  were exposed to a three hour humidification step carried out at a relative humidity of 90%. Spores were then subjected to a deep vacuum of at least 29 inches of water, then were exposed to 1,000 ppm chlorine dioxide gas for four hours. In some cases, the spores were subjected to multiple treatment cycles. Spores were then cultured in thioglycollate broth to determine whether they were viable following the decontamination protocol. Table 4 shows the effects of successive ClO<sub>2</sub> treatment cycles on decontamination efficacy.

20

**Table 4: Consecutive ClO<sub>2</sub> Treatments**

Indicator	Cycle 1	Cycle 2	Cycle 3
10 <sup>10</sup> Weaponized Spores	$5.7 \times 10^4$	0	$3.3 \times 10^1$
TP 10	$4.3 \times 10^5$	$1.3 \times 10^3$	$7.2 \times 10^2$
10 <sup>10</sup> Weaponized Spores + Control	$1.8 \times 10^{10}$	$1.8 \times 10^{10}$	$1.8 \times 10^{10}$
TP 10 + Control	$1.9 \times 10^{10}$	$1.9 \times 10^{10}$	$1.9 \times 10^{10}$

Thus, exposing spore-contaminated articles to a deep vacuum prior to application of chlorine effect of exposure to a deep vacuum and consecutive treatment cycles on decontamination efficacy.

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**Example 7: Effect of exposure time and chlorine dioxide concentration on decontamination efficacy**

This example shows that increasing the exposure time to chlorine dioxide gas 5 increases decontamination efficacy, that longer exposure times are required for spores contained in an envelope than for free spores, and that longer exposure times are required for weaponized spores than for non-weaponized spores.

Weaponized spores or conventional bioindicator spores (MS) at a concentration of  $10^{10}$  were either prepared as free spores or confined to glassine envelopes. Spores 10 were then exposed to a three hour humidification step carried out at a relative humidity of 90%, and were then subjected to a deep vacuum of at least 29 inches of water. Spores were then exposed to 1,000 ppm chlorine dioxide gas for 0.5 to 6 hours (See Tables 5- 16, below). Spores were then plated in serial dilutions on agar plates and the resulting colonies were counted to determine decontamination efficacy. In 15 addition, spores were cultured in thioglycollate broth for 24-48 hours to determine whether they were viable following the decontamination protocol. Tables 5-16 show the effects of chlorine dioxide exposure time on decontamination of free spores versus spores in envelopes, and the differences in decontamination efficacy on weaponized spores versus conventional bioindicator spores. The results shown in Tables 5-16 are 20 summarized in FIG. 5.

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**Table 5: Decontamination efficacy of 0.5 hour incubation of spores without glassine envelopes**

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	-	-
MS <sup>10</sup> 2	0	0	0	-	-
MS <sup>10</sup> 3	0	0	0	-	-
MS <sup>10</sup> 4	0	0	0	-	-
MS <sup>10</sup> 5	0	0	0	-	-
$10^{10}$ Weaponized Spores 1	870	87	0	+	+
$10^{10}$ Weaponized Spores 2	410	57	3	+	+
$10^{10}$ Weaponized Spores 3	1090	97	10	+	+
$10^{10}$ Weaponized Spores 4	533	27	0	+	+
$10^{10}$ Weaponized Spores 5	1527	13	3	+	+
PBS - Control	0	0	0	-	-

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**Table 6: Decontamination efficacy of 0.5 hour incubation of spores in glassine envelopes**

Envelope	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	$10^{-4}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	TNTC	TNTC	TNTC	+	+
MS <sup>10</sup> 2	TNTC	TNTC	1460	+	+
MS <sup>10</sup> 3	0	0	0	+	+
MS <sup>10</sup> 4	TNTC	TNTC	TNTC	+	+
	$10^{-6}$ (Dilution on agar)	$10^{-7}$ (Dilution on agar)	$10^{-8}$ (Dilution on agar)		
$10^{10}$ Weaponized Spores 1	TNTC	3427	40	+	+
$10^{10}$ Weaponized Spores 2	TNTC	TNTC	960	+	+
$10^{10}$ Weaponized Spores 3	TNTC	2023	180	+	+
$10^{10}$ Weaponized Spores 4	TNTC	TNTC	687	+	+-
PBS – Control	0	0	0	-	-

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**Table 7: Decontamination efficacy of 1.0 hour incubation of spores without glassine envelopes**

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	-	-
MS <sup>10</sup> 2	0	0	0	-	-
MS <sup>10</sup> 3	0	0	0	-	-
MS <sup>10</sup> 4	3	0	0	+	+
MS <sup>10</sup> 5	0	0	0	-	-
$10^{10}$ Weaponized Spores 1	0	0	0	-	-
$10^{10}$ Weaponized Spores 2	1873	103	30	+	+
$10^{10}$ Weaponized Spores 3	0	13	0	+	+
$10^{10}$ Weaponized Spores 4	7	3	3	+	+
$10^{10}$ Weaponized Spores 5	3	0	0	+	+
PBS - Control	0	0	0	-	-

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**Table 8: Decontamination efficacy of 1.0 hour incubation of spores in glassine envelopes**

Envelope	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	$10^{-4}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	ND	ND	+	+
MS <sup>10</sup> 2	0	ND	ND	+	+
MS <sup>10</sup> 3	0	ND	ND	+	+
MS <sup>10</sup> 4	2100	ND	ND	+	+
	$10^{-4}$ (Dilution on agar)	$10^{-5}$ (Dilution on agar)	$10^{-6}$ (Dilution on agar)		
$10^{10}$ Weaponized Spores 1	TNTC	3427	40	+	+
$10^{10}$ Weaponized Spores 2	TNTC	TNTC	960	+	+
$10^{10}$ Weaponized Spores 3	TNTC	2023	180	+	+
$10^{10}$ Weaponized Spores 4	TNTC	TNTC	687	+	+-
PBS – Control	0	0	0	-	-

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**Table 9: Decontamination efficacy of 2.0 hour incubation of spores in glassine envelopes**

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	TNTC	TNTC	TNTC	+	+
MS <sup>10</sup> 2	0	0	0	+	+
MS <sup>10</sup> 3	0	0	0	+	+
MS <sup>10</sup> 4	0	0	0	+	+
	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)		
$10^{10}$ Weaponized Spores 1	0	0	0	+	+
$10^{10}$ Weaponized Spores 2	TNTC	TNTC	4350	+	+
$10^{10}$ Weaponized Spores 3	3203	463	53	+	+
$10^{10}$ Weaponized Spores 4	0	0	0	+	+
PBS - Control	0	0	0	-	-

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**Table 10: Decontamination efficacy of 2.0 hour incubation of spores without glassine envelopes**

Envelope	10 <sup>-1</sup> (Dilution on agar)	10 <sup>-2</sup> (Dilution on agar)	10 <sup>-3</sup> (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	-	-
MS <sup>10</sup> 2	0	0	0	-	-
MS <sup>10</sup> 3	0	0	0	+	+
MS <sup>10</sup> 4	0	0	0	-	-
MS <sup>10</sup> 5	0	0	0	-	-
10 <sup>10</sup> Weaponized Spores 1	0	0	0	-	-
10 <sup>10</sup> Weaponized Spores 2	0	0	0	-	-
10 <sup>10</sup> Weaponized Spores 3	0	0	0	-	-
10 <sup>10</sup> Weaponized Spores 4	0	0	0	-	+
10 <sup>10</sup> Weaponized Spores 5	0	0	0	-	-
PBS - Control	0	0	0	-	-

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**Table 11: Decontamination efficacy of 3.0 hour incubation of spores without glassine envelopes**

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	-	-
MS <sup>10</sup> 2	0	0	0	-	-
MS <sup>10</sup> 3	0	0	0	-	-
MS <sup>10</sup> 4	0	0	0	-	-
MS <sup>10</sup> 5	0	0	0	-	-
$10^{10}$ Weaponized Spores 1	0	0	0	-	-
$10^{10}$ Weaponized Spores 2	0	0	0	-	-
$10^{10}$ Weaponized Spores 3	0	0	0	-	-
$10^{10}$ Weaponized Spores 4	0	0	0	-	-
$10^{10}$ Weaponized Spores 5	0	0	0	-	-
PBS – Control	0	0	0	-	-

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**Table 12: Decontamination efficacy of 4.0 hour incubation of spores without glassine envelopes**

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	-	-
MS <sup>10</sup> 2	0	0	0	-	-
MS <sup>10</sup> 3	0	0	0	-	-
MS <sup>10</sup> 4	0	0	0	-	-
MS <sup>10</sup> 5	0	0	0	-	-
$10^{10}$ Weaponized Spores 1	0	0	0	-	-
$10^{10}$ Weaponized Spores 2	0	0	0	-	-
$10^{10}$ Weaponized Spores 3	0	0	0	-	-
$10^{10}$ Weaponized Spores 4	0	0	0	-	-
$10^{10}$ Weaponized Spores 5	0	0	0	-	-
PBS – Control	0	0	0	-	-

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**Table 13: Decontamination efficacy of 4.0 hour incubation of spores in glassine envelopes**

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	+	+
MS <sup>10</sup> 2	0	0	0	+	+
MS <sup>10</sup> 3	0	0	0	+	+
MS <sup>10</sup> 4	0	0	0	+	+
	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)		
$10^{10}$ Weaponized Spores 1	0	0	0	+	+
$10^{10}$ Weaponized Spores 2	10	0	10	+	+
$10^{10}$ Weaponized Spores 3	0	0	0	+	+
$10^{10}$ Weaponized Spores 4	0	0	0	+	+
PBS – Control	0	0	0	-	-

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**Table 14: Decontamination efficacy of 5.0 hour incubation of spores without glassine envelopes**

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	-	-
MS <sup>10</sup> 2	0	0	0	-	-
MS <sup>10</sup> 3	0	0	0	-	-
MS <sup>10</sup> 4	0	0	0	-	-
MS <sup>10</sup> 5	0	0	0	-	-
$10^{10}$ Weaponized Spores 1	0	0	0	-	-
$10^{10}$ Weaponized Spores 2	0	0	0	-	-
$10^{10}$ Weaponized Spores 3	0	0	0	-	-
$10^{10}$ Weaponized Spores 4	0	0	0	-	-
$10^{10}$ Weaponized Spores 5	0	0	0	-	-
PBS - Control	0	0	0	-	-

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**Table 15: Decontamination efficacy of 6.0 hour incubation of spores without glassine envelopes**

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	-	-
MS <sup>10</sup> 2	0	0	0	-	-
MS <sup>10</sup> 3	0	0	0	-	-
MS <sup>10</sup> 4	0	0	0	-	-
MS <sup>10</sup> 5	0	0	0	-	-
$10^{10}$ Weaponized Spores 1	0	0	0	-	-
$10^{10}$ Weaponized Spores 2	0	0	0	-	-
$10^{10}$ Weaponized Spores 3	0	0	0	-	-
$10^{10}$ Weaponized Spores 4	0	0	0	-	-
$10^{10}$ Weaponized Spores 5	0	0	0	-	-
PBS - Control	0	0	0	-	-

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Table 16: Decontamination efficacy of 6.0 hour incubation of spores in glassine envelopes

Envelope	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)	Broth 24 Hrs.	Broth 48 Hrs.
MS <sup>10</sup> 1	0	0	0	+	+
MS <sup>10</sup> 2	0	0	0	+	+
MS <sup>10</sup> 3	0	0	0	+	+
MS <sup>10</sup> 4	0	0	0	+	+
	$10^{-1}$ (Dilution on agar)	$10^{-2}$ (Dilution on agar)	$10^{-3}$ (Dilution on agar)		
$10^{10}$ Weaponized Spores 1	0	0	0	+	+
$10^{10}$ Weaponized Spores 2	10	0	10	+	+
$10^{10}$ Weaponized Spores 3	0	0	0	+	+
$10^{10}$ Weaponized Spores 4	0	0	0	+	+
PBS – Control	0	0	0	-	-

5 Thus, increasing the exposure time to chlorine dioxide gas increases decontamination efficacy, longer exposure times are required for spores contained in an envelope than for free spores, and longer exposure times are required for weaponized spores than for non-weaponized spores.

10 This disclosure provides methods and apparatus for decontaminating a porous article. It will be apparent that the precise details of the methods and apparatus described may be varied or modified without departing from the spirit of the described

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disclosure. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

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## CLAIMS

We claim:

1. A method of decontaminating a porous article, wherein the method comprises:
  - 5 enclosing the article in an environment;
  - humidifying the environment to enhance the susceptibility of the spores to subsequent decontamination with chlorine dioxide;
  - reducing the pressure in the humidified environment to at least as low as 100 inches of water ( $0.25396 \text{ kg/cm}^2$ ); and
  - 10 then introducing into the environment a concentration of gaseous chlorine dioxide effective to decontaminate the article by killing substantially 100% of the spores.
2. The method of claim 1, wherein the environment is a rigid container, autoclave,
  - 15 or hyperbaric chamber.
3. The method of claim 1, wherein the environment is a sealed room or building.
4. The method of claim 3, wherein enclosing the article in an environment
  - 20 comprises sealing the room or building.
5. The method of claim 4, wherein the method further comprises reinforcing a window prior to reducing the pressure in the environment.
- 25 6. The method of claim 1, wherein humidifying the environment comprises increasing the relative humidity of the environment to at least 95%.
7. The method of claim 6, wherein humidifying the environment comprises

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increasing the relative humidity of the environment to at least 90% for at least one hour.

8. The method of claim 7, wherein humidifying the environment comprises increasing the relative humidity of the environment to at least 90% for at least three hours.

9. The method of claim 1, wherein the pressure in the humidified environment is reduced to at least as low as 50 inches of water ( $0.12698 \text{ kg/cm}^2$ ).

10 10. The method of claim 1, wherein the pressure in the humidified environment is reduced to at least as low as 29 inches of water ( $0.0736484 \text{ kg/cm}^2$ ).

11. The method of claim 1, wherein the concentration of gaseous chlorine dioxide is at least 1000 parts per million.

15 12. The method of claim 1, wherein the concentration of gaseous chlorine dioxide is at least 2500 parts per million.

20 13. The method of claim 1, wherein the article is exposed to the gaseous chlorine dioxide for at least one hour.

14. The method of claim 13, wherein the article is exposed to the gaseous chlorine dioxide for at least three hours.

25 15. The method of claim 14, wherein the article is exposed to the gaseous chlorine dioxide for at least six hours.

16. The method of claim 1, wherein the spore is a *Bacillus anthracis* spore.

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17. The method of claim 1, wherein the spore is a weaponized spore.
18. The method of claim 1, wherein the article comprises paper.  
5
19. The method of claim 1, wherein the environment is a decontamination chamber, humidifying the environment comprises increasing the relative humidity of the environment to at least 90% for at least one hour, the pressure in the humidified environment is reduced to at least as low as 29 inches of water ( $0.0736484 \text{ kg/cm}^2$ ), the concentration of gaseous chlorine dioxide is at least 1000 parts per million, and the article is exposed to the gaseous chlorine dioxide for at least one hour.  
10
20. The method of claim 1, wherein the environment is a room, enclosing the article in an environment comprises sealing the room, humidifying the environment comprises increasing the relative humidity of the environment to at least 90% for at least one hour, the pressure in the humidified environment is reduced to at least as low as 50 inches of water ( $0.12698 \text{ kg/cm}^2$ ), the concentration of gaseous chlorine dioxide is at least 1000 parts per million, and the article is exposed to the gaseous chlorine dioxide for at least one hour.  
15
21. The method of claim 1, wherein the environment is a building, enclosing the article in an environment comprises sealing the building, humidifying the environment comprises increasing the relative humidity of the environment to at least 90% for at least one hour, the pressure in the humidified environment is reduced to at least as low as 50 inches of water ( $0.12698 \text{ kg/cm}^2$ ), the concentration of gaseous chlorine dioxide is at least 1000 parts per million, the article is exposed to the gaseous chlorine dioxide for at least one hour, and the method further comprises reinforcing a window prior to reducing the pressure in the environment.  
20
- 25

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22. A method of decontaminating a porous article, wherein the method comprises:
  - enclosing the article in a decontamination chamber;
  - increasing the relative humidity in the decontamination chamber to at least 95%;
  - reducing the pressure in the humidified decontamination chamber to at least as low as 50 inches of water ( $0.12698 \text{ kg/cm}^2$ ); and
  - then introducing into the decontamination chamber at least 1000 parts per million gaseous chlorine dioxide, thus decontaminating the article by killing substantially 100% of the spores.
- 10 23. A method of decontaminating a porous article, wherein the method comprises:
  - enclosing the article in a sealed room or building;
  - increasing the relative humidity in the sealed room or building to at least 95%;
  - reducing the pressure in the humidified room or building to at least as low as 100 inches of water ( $0.25396 \text{ kg/cm}^2$ ); and
  - then introducing into the room or building at least 1000 parts per million gaseous chlorine dioxide, thus decontaminating the article by killing substantially 100% of the spores
- 20 24. An apparatus for decontaminating a porous article, wherein the apparatus comprises:
  - a selectively sealable decontamination chamber;
  - a decontamination chamber humidifier;
  - a source of chlorine dioxide gas in fluid communication with the decontamination chamber; and
  - a decontamination chamber vacuum generator.
- 25 25. The apparatus of claim 24, further comprising:

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a first fluid flow path for transferring humidified gas from the decontamination chamber humidifier to the selectively sealable decontamination chamber;

a second fluid flow path for transferring chlorine dioxide gas from the source of chlorine dioxide to the selectively sealable decontamination chamber; and

5 a third fluid flow path for evacuating the selectively sealable decontamination chamber via the decontamination chamber vacuum generator.

26. The apparatus of claim 25, further comprising a flow regulator in the first fluid flow path.

10 27. The apparatus of claim 25, further comprising a rotometer in the first fluid flow path.

28. The apparatus of claim 25, further comprising a nitrogen source and a fourth fluid flow path for transferring nitrogen gas to the decontamination chamber humidifier.

15 29. The apparatus of claim 28, further comprising a fill valve in the fourth fluid flow path.

20 30. The apparatus of claim 28, further comprising a flow regulator in the fourth fluid flow path.

31. The apparatus of claim 25, further comprising a flow regulator in the third fluid flow path.

25 32. The apparatus of claim 25, further comprising a ventilation valve in the second fluid flow path.

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33. The apparatus of claim 24, wherein the source of chlorine dioxide gas is a chlorine dioxide generator.
34. The apparatus of claim 24, wherein the selectively sealable decontamination chamber is a rigid container.  
5
35. The apparatus of claim 24, wherein the apparatus further comprises a heat source for providing heat to the selectively sealable decontamination chamber.
- 10 36. The apparatus of claim 24, wherein the apparatus further comprises a hygrometer for regulating humidity in the selectively sealable decontamination chamber.
- 15 37. The apparatus of claim 34, wherein the rigid container comprises a heat source, a thermostat for regulating the heat source, and a hygrometer for regulating humidity in the rigid container.
- 20 38. The apparatus of claim 24, wherein the selectively sealable decontamination chamber comprises an autoclave.
39. The apparatus of claim 24, wherein the selectively sealable decontamination chamber comprises a hyperbaric chamber.
- 25 40. The apparatus of claim 24, wherein the decontamination chamber comprises a room or building.
41. The apparatus of claim 40, where the room or building is a sealed room or building that forms a sealed environment.

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42. The apparatus of claim 41, where the room or building comprises a window and the window is reinforced to withstand a vacuum pressure of at least as low as 100 inches of water ( $0.25396 \text{ kg/cm}^2$ ).

5

43. The apparatus of claim 41, where the room or building comprises a window and the window has been reinforced to withstand a vacuum pressure of at least as low as 50 inches of water ( $0.12698 \text{ kg/cm}^2$ ).

10 44. The apparatus of claim 41, where the room or building comprises a window and the window has been reinforced to withstand a vacuum pressure of at least as low as 29 inches of water ( $0.0736484 \text{ kg/cm}^2$ ).

15 45. The apparatus of claim 41, wherein the apparatus further comprises a heat source for providing heat to the room or building.

46. The apparatus of claim 41, wherein the apparatus further comprises a hygrometer for regulating humidity in the selectively sealable decontamination chamber.

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## METHOD AND APPARATUS FOR BIOWEAPON DECONTAMINATION

### ABSTRACT OF THE DISCLOSURE

The present disclosure relates to the decontamination of articles contaminated  
5 with bioweapons, particularly methods and apparatus for decontaminating articles  
contaminated with sporulated bioweapons. In some embodiments, the methods are  
methods of decontaminating an environment, for example a room or building  
contaminated with a sporulated bioweapon.

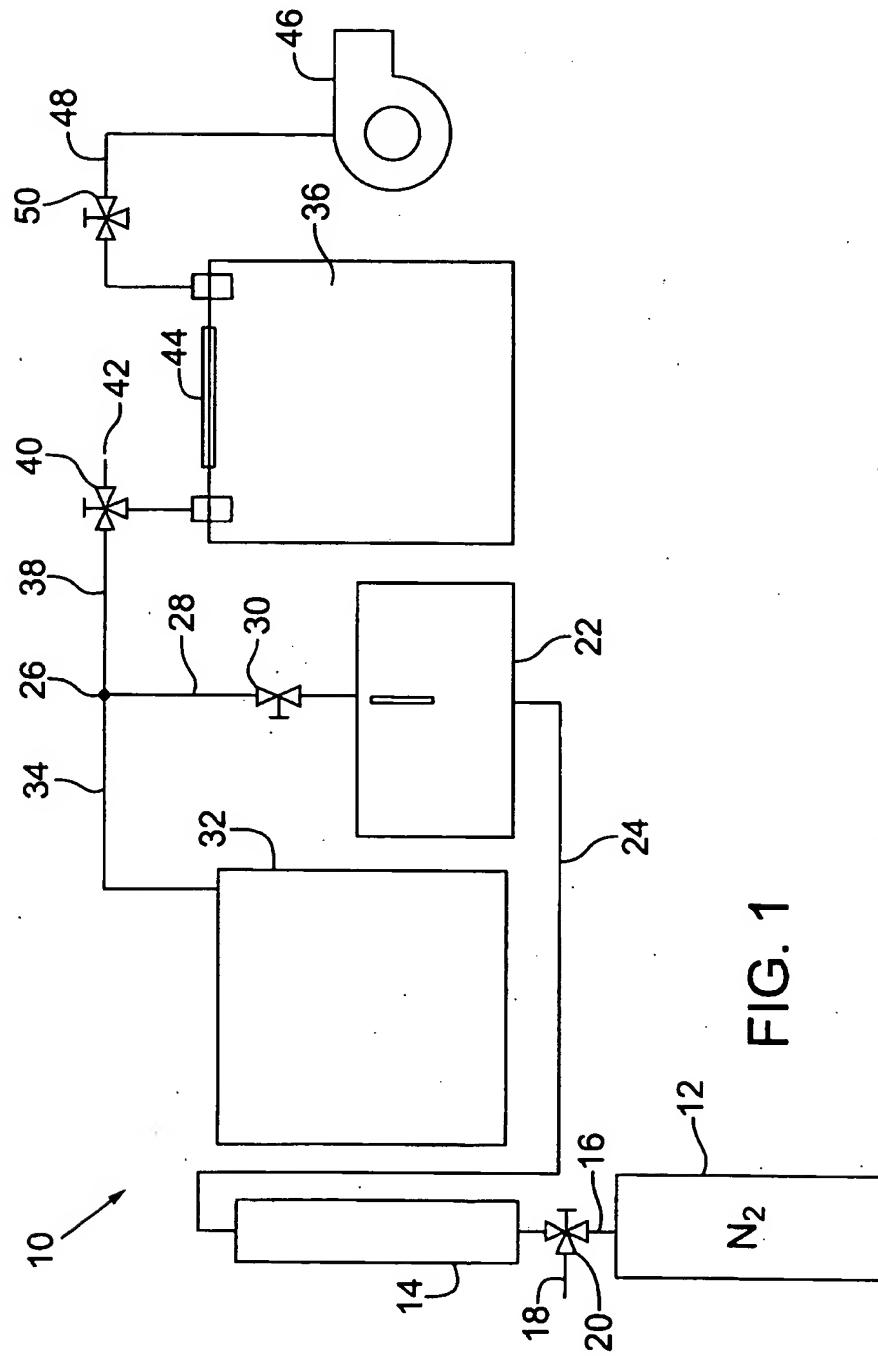


FIG. 1

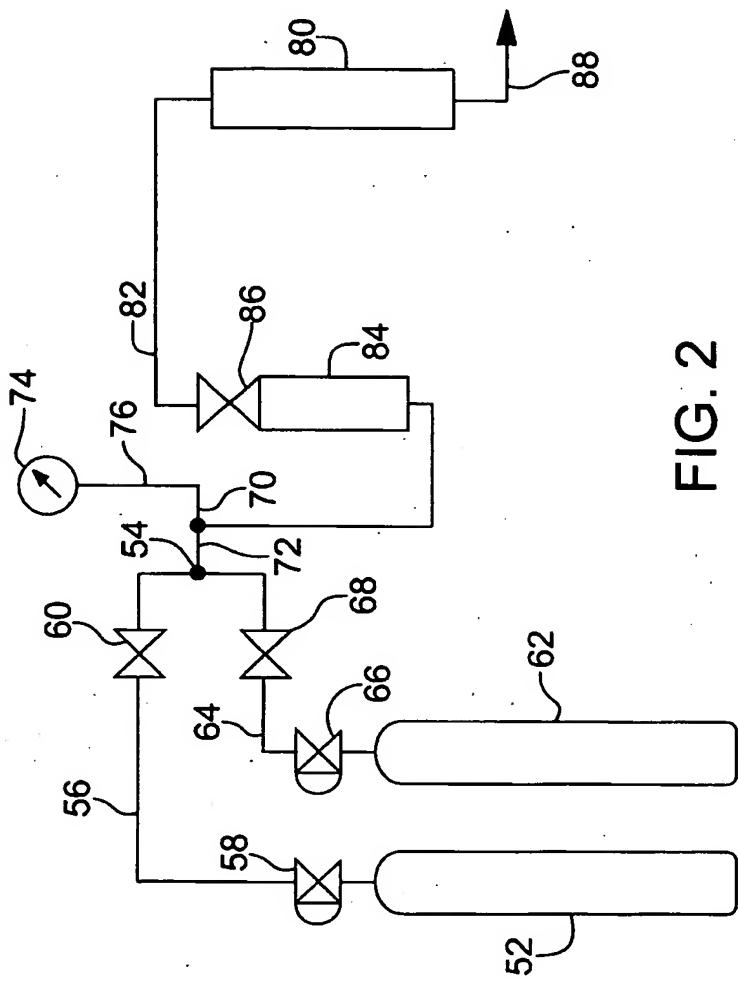


FIG. 2

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Title: METHOD AND APPARATUS FOR BIOWEAPON  
DECONTAMINATION  
Attorney's Matter No.: 4239-64793/WDN/GLB:kam  
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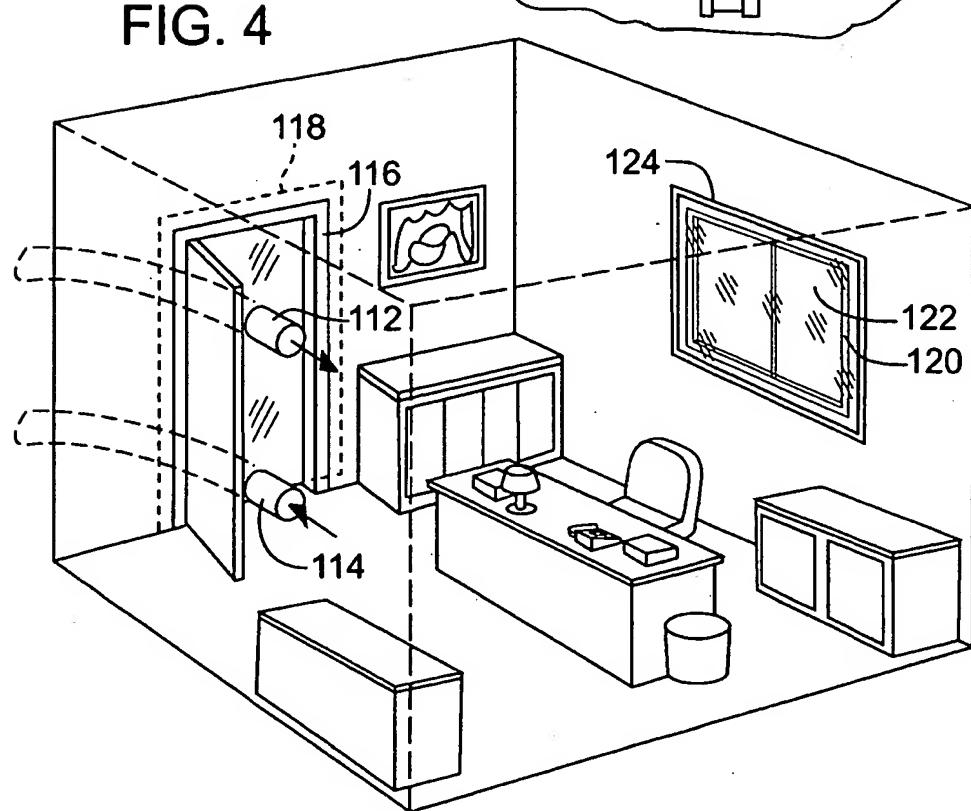
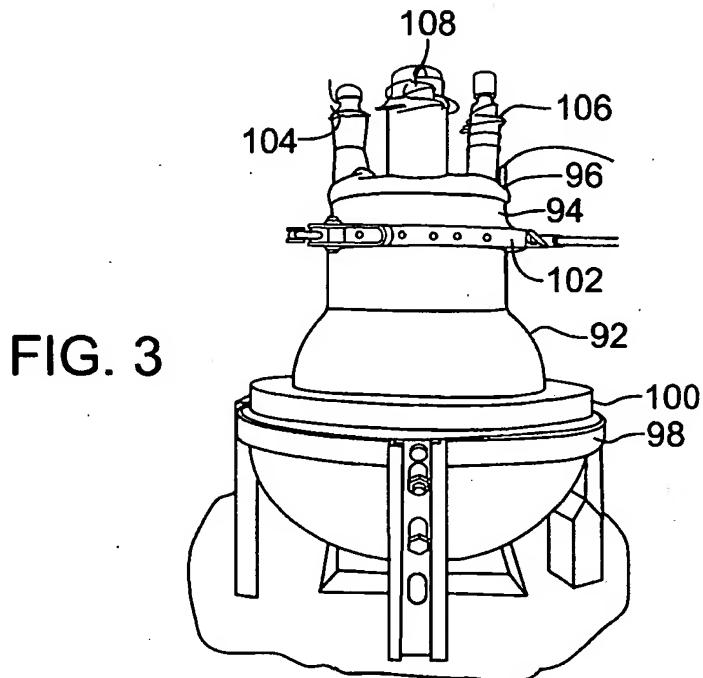


FIG. 5 Number Organisms Recovered after treatment with  
1000 ppm ClO<sub>2</sub>-in glassine

